

## Article

# Experience recruits MSK1 to expand the dynamic range of synapses and enhance cognition

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**Experience recruits MSK1 to expand the dynamic range of synapses and enhance cognition**

Abbreviated title: MSK1 regulates the dynamic range of synapses and cognition

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40

41



42 **ABSTRACT**

43 Experience powerfully influences neuronal function and cognitive performance, but the cellular and  
44 molecular events underlying the experience-dependent enhancement of mental ability have  
45 remained elusive. In particular, the mechanisms that couple the external environment to the genomic  
46 changes underpinning this improvement are unknown. To address this we have used male mice  
47 harbouring an inactivating mutation of mitogen- and stress-activated protein kinase 1 (MSK1), a  
48 BDNF-activated enzyme downstream of the MAPK pathway. We show that MSK1 is required for the  
49 full extent of experience-induced improvement of spatial memory, for the expansion of the dynamic  
50 range of synapses, exemplified by the enhancement of hippocampal LTP and LTD, and for the  
51 regulation of the majority of genes influenced by enrichment. In addition, and unexpectedly, we show  
52 that experience is associated with an MSK1-dependent downregulation of key MAPK and plasticity-  
53 related genes, notably of *EGR1/Zif268* and *Arc/Arg3.1*, suggesting the establishment of a novel  
54 genomic landscape adapted to experience. By coupling experience to homeostatic changes in gene  
55 expression MSK1, represents a prime mechanism through which the external environment has an  
56 enduring influence on gene expression, synaptic function and cognition.

57

58

59 **SIGNIFICANCE STATEMENT**

60 Our everyday experiences strongly influence the structure and function of the brain. Positive  
61 experiences encourage the growth and development of the brain and support enhanced learning and  
62 memory and resistance to mood disorders such as anxiety. While this has been known for many  
63 years, how this occurs is not clear. Here we show that many of the positive aspects of experience  
64 depend upon an enzyme called MSK1. Using male mice with a mutation in MSK1 we show that MSK1  
65 is necessary for the majority of gene expression changes associated with experience, extending the  
66 range over which the communication between neurons occurs, and for both the persistence of  
67 memory and the ability to learn new task rules.

68 **INTRODUCTION**

69 Experience exerts a profound influence on the structure and function of the mammalian brain (Kolb  
70 and Whishaw, 1998). While this was predicted by early physiologists (von Bernhardi et al., 2017), it  
71 was the pioneering work of Donald Hebb in the 1940s that first demonstrated the enhanced cognitive  
72 abilities of rats raised in the stimulating environment of his home compared to their counterparts  
73 reared in a laboratory (Hebb, 1947, 1949). Subsequent studies have identified many cellular changes  
74 associated with the enhanced cognition that arises following exposure to larger social groups, a  
75 complex environment and exercise wheels, including neurogenesis, greater dendritic spine density  
76 and enhanced synaptic plasticity (Rosenzweig and Bennett, 1996; Sale et al., 2014; Ohline and  
77 Abraham, 2019). In experimental animals these cellular adaptations translate into enhanced cognition  
78 (Sale et al., 2014), reduced anxiety (Rogers et al., 2019), the acceleration of recovery from brain injury  
79 (de la Tremblaye et al., 2019), resistance to drugs of abuse (Stairs and Bardo, 2009) and the alleviation  
80 of signs associated with animal models of Parkinson's disease (Wassouf and Schulze-Hentrich, 2019),  
81 Huntington's disease (Mo et al., 2015) and autism spectrum disorder (Gubert and Hannan, 2019).  
82 Indeed, parallels have been described in humans, including for children with autism (Woo and Leon,  
83 2013; Woo et al., 2015; Aronoff et al., 2016), in terms of the benefits for mental health and wellbeing,  
84 and brain structure and function, of social interaction, skills training, exercise and higher  
85 socioeconomic status (Kolb and Whishaw, 1998; Sale et al., 2014; Farah, 2018; Miguel et al., 2019;  
86 Rogers et al., 2019).

87

88 However, despite the importance of establishing how experience influences brain function, the  
89 intracellular signalling cascade and the enduring influence on the genome that underlie these cellular  
90 and cognitive adaptations to enriched environments have yet to be identified. An ideal candidate to

91 mediate such a coupling between the environment and the genome would be positioned between  
92 the BDNF TrkB receptors repeatedly implicated in mediating the benefits of environmental  
93 enrichment (Cowansage et al., 2010; Sale et al., 2014; Rogers et al., 2019), and the genomic changes  
94 required for persistent modifications to neuronal structure, synaptic function and learning and  
95 memory (Alberini and Kandel, 2014; Takeuchi et al., 2014).

96  
97 The nuclear kinase, mitogen- and stress-activated protein kinase 1 (MSK1), is well-placed to transduce  
98 the sensory experiences associated with enrichment into the enduring cellular, molecular and  
99 genomic events underpinning enhanced cognition. MSK1 is activated by BDNF and regulates gene  
100 expression, notably via the phosphorylation of CREB (Arthur et al., 2004; Reyskens and Arthur, 2016),  
101 and including that of the plasticity-related protein Arc/Arg3.1 (Hunter et al., 2017). In addition, MSK1  
102 is expressed in hippocampal neurons (Heffron and Mandell, 2005; Sindreu et al., 2007), a major site of  
103 the effects of environmental enrichment (Hirase and Shinohara, 2014). Using mice harbouring a  
104 knock-in point mutation of the MSK1 gene that results in the elimination of the kinase activity of  
105 MSK1 (kinase dead; MSK1 KD), but which does not affect hippocampal learning and memory or  
106 synaptic plasticity under standard housing conditions (Daumas et al., 2017), we previously showed  
107 that the kinase activity of MSK1 was required for homeostatic synaptic scaling in vitro, and the in vivo  
108 enrichment-induced enhancement of miniature excitatory postsynaptic currents (Corrêa et al., 2012;  
109 Lalo et al., 2018). However, this left unanswered the important question of the genomic, plasticity  
110 and cognitive implications of these isolated observations at the synapse.

111  
112 Using wild-type (WT) and MSK1 KD mice we have found that the kinase activity of MSK1 is necessary  
113 for the full benefits of enrichment on cognition, in particular, in the persistence of hippocampal

114 spatial memory and cognitive flexibility. As a potential cellular correlate of this enhanced cognition,  
115 we discovered that enrichment is associated with an MSK1-dependent expansion of the dynamic  
116 range of synapses: both hippocampal long-term potentiation (LTP) and long-term depression (LTD)  
117 are enhanced, thereby allowing synapses to code a greater amount of information. Finally, an RNA-  
118 Seq analysis of the hippocampal transcriptome under standard and enriched conditions revealed a  
119 predominant requirement for MSK1 in the experience-dependent regulation of gene expression.  
120 Moreover, we observed an unexpected and MSK1-dependent downregulation of plasticity-associated  
121 proteins and transcription factors such as Arc/Arg3.1 and EGR1. These observations suggest that  
122 MSK1 couples the external environment to the genome, and through this coupling initiates both the  
123 cellular and molecular events leading to synaptic and cognitive enhancement, and an experience-  
124 dependent genomic homeostasis designed to maintain the stability of the enhanced brain.

125

## 126 MATERIALS AND METHODS

### 127 Animals

128 The MSK1 kinase dead (KD) mouse used in this study has been described previously (Corrêa et al.,  
 129 2012). Briefly, Asp194 in the endogenous MSK1 gene was mutated to Ala (D194A). This inactivates the  
 130 N-terminal kinase domain of MSK1. Genotyping was carried out by PCR using the primers 5'-  
 131 CGGCCATGTGGTGCTGACAGC-3' and 5'-GGGTCAGAGGCCTGCACTAGG-3', which gives 378 bp and 529  
 132 bp products for WT and targeted alleles, respectively. All the mice used in this study were on a C57-  
 133 Bl/6J genetic background after at least four backcrosses from the original C57-Bl/6n strain used by  
 134 Taconic Artemis to generate the mutant mice. Male WT C57-Bl/6J mice purchased from Charles River  
 135 UK were used for backcrossing with female MSK1 KD homozygous mutants. The mice used in this  
 136 study were maintained as homozygous and WT lines derived from founder homozygous and WT  
 137 breeders from an initial series of heterozygote crosses. Subsequent backcrossing occurred when the  
 138 founder mice had come to the end of their reproductive lifetime (typically three litters). This strategy  
 139 avoided genetic divergence of the two lines. While using WT and homozygous mutant littermates  
 140 from heterozygote crosses is experimentally desirable, our breeding strategy is appropriate when  
 141 homozygous mutants of both sexes are viable and fertile (Jax, 2009), allowed large numbers of  
 142 animals of the correct age, genotype, housing condition and sex to be bred in order that experiments  
 143 could be conducted in time-limited batches, minimizing variability. Our breeding strategy also avoided  
 144 the unnecessary breeding and culling of large numbers of heterozygote mice (50% of all litters) in  
 145 keeping with the drive to reduce the number of animals used in research, and with institutional and  
 146 funder expectations. We note that many experimental parameters were similar between WT and  
 147 MSK1 KD mice under standard and enriched housing conditions, and that the hippocampal expression  
 148 of only three genes differed between the two genotypes under standard housing conditions. This

149 suggests that the breeding strategy did not introduce confounds that could have affected our  
150 observations.

151

152 Mice were maintained under a 12/12 light dark cycle (lights on at 7.00 am) in a facility kept at 20-24  
153 °C and were given ad libitum access to standard mouse chow and water. All animal procedures  
154 conformed with local, national and EU guidelines concerning the welfare of experimental animals.  
155 Behavioral studies were performed under the auspices of Home Office licence PPL 70/7821 granted to  
156 BGF. Male mice were used in this study to facilitate comparison with previous studies on MSK1 KD  
157 mice (Correa et al., 2012; Daumas et al., 2017). The mice have been deposited with the  
158 INFRAFRONTIER/EMMA repository at MRC Harwell, UK (<https://www.har.mrc.ac.uk>).

159

#### 160 **Environmental enrichment**

161 Environmental enrichment was provided via the rearing of WT and MSK1 KD mice in large individually  
162 ventilated rat cages (Tecniplast 1500U; 480 x 375 x 210 mm; 1500 cm<sup>2</sup> floor area) containing bedding  
163 material, a cardboard tube, one running wheel and several plastic toys (tunnels, platforms, see-saws)  
164 and a metal ladder. To provide novelty, toys were moved around twice per week and new toys  
165 introduced once per week. Two to three pregnant dams (E14-15, based on vaginal plugs) were  
166 randomly selected and placed in enriched cages to provide additional mothering (D'Amato et al.,  
167 2010) and larger groups for social interactions. Dams typically gave birth within 1 – 2 days of each  
168 other. At weaning (P23-24), all females were removed and the males (typically 8) remained in the  
169 enriched environment for the remainder of the experimental period (to ~5 months of age). Age-  
170 matched male mice were born and maintained in standard housing (Tecniplast 1284L; 365 x 207 x 140  
171 mm; 530 cm<sup>2</sup> floor area; two to four mice with bedding material and a cardboard tube) and served as

172 controls for the environmental enrichment groups. Cage cleaning was done on Mondays for all  
173 standard and enriched cages. Toys in enriched cages were changed on Tuesdays and were moved  
174 around the enriched cages on Mondays and Thursdays. To keep disruption of the home environment  
175 to a minimum, sawdust and bedding were never changed at the same time as toys. To minimise  
176 disruptions to established hierarchies, during cage cleaning and behavioural testing all mice (standard  
177 and enriched) were removed to a different cage (standard cage size with one toy from the enriched  
178 cage for enriched mice) and then were returned together. This was effective in reducing within-cage  
179 aggression between the males.

180

#### 181 **Analysis of dendritic spine density**

182 Male WT and MSK1 KD mice were killed at 17-18 weeks of age, two weeks after the end of  
183 behavioural testing, by cervical dislocation in accordance with the UK Animals (Scientific Procedures)  
184 Act 1986 and with local Animal Welfare and Ethical Review Board approval. The brains were removed  
185 and processed with the FD Rapid Golgi Stain kit (FD NeuroTechnologies, Inc.) in accordance with the  
186 manufacturer's protocol. Impregnated brains (4 per group) were sectioned with a vibratome (coronal  
187 sections; 200  $\mu$ m thick) stained and mounted. Dendritic spines on the secondary branches of apical  
188 dendrites of hippocampal CA1 neurons were counted. Spine count was conducted blind to genotype  
189 and housing condition. ImageJ software was used to measure dendritic length and the numbers of  
190 spines on each dendritic segment. Images for spine density analysis were captured with a 40x  
191 objective on a Zeiss Imager 2 AXIO microscope.

192

#### 193 **Hippocampal slice preparation and extracellular recordings**



194 Male WT and MSK1 KD mice (3-5 months old) were killed by cervical dislocation in accordance with  
195 the UK Animals (Scientific Procedures) Act 1986 and with local Animal Welfare and Ethical Review  
196 Board approval. Hippocampal slices (400  $\mu\text{m}$ ) were cut in ice cold aCSF using either a Stoelting tissue  
197 chopper or a Microm HM650V tissue slicer. Upon cutting, slices were transferred to a recording  
198 chamber and placed upon a mesh support at the interface of an oxygen-rich atmosphere and  
199 underlying aCSF where they remained for the duration of the experiment, which typically started  
200 some 2 hours after slice cutting. The temperature of the aCSF was set at 31°C and the flow rate was  
201 1.5 ml/min. The aCSF used for the preparation, maintenance and recording of slices contained in mM:  
202 124.0 NaCl, 4.4 KCl, 1.0  $\text{Na}_2\text{HPO}_4$ , 25.0  $\text{NaHCO}_3$ , 2.0  $\text{CaCl}_2$ , 2.0  $\text{MgCl}_2$ , 10.0 D-glucose. aCSF was  
203 bubbled with 95 %  $\text{O}_2$ /5 %  $\text{CO}_2$ ; pH 7.4. All salts used in the aCSF were obtained from either Fisher  
204 Scientific or Sigma-Aldrich.

205

206 To make extracellular recordings of field excitatory postsynaptic potentials (fEPSPs), an aCSF-filled  
207 glass microelectrode was placed in stratum radiatum of area CA1 and two concentric bipolar  
208 stimulating electrodes (CBBRC75, FHC) were placed either side of the recording electrode. This  
209 allowed alternating recordings to be made from two independent but convergent afferent Schaffer  
210 collateral/commissural fibre pathways. Each pathway was stimulated every 90 s with a monophasic  
211 pulse of 0.1 ms duration. Pathway-independence was assessed via a crossed paired-pulse facilitation  
212 protocol (at 50 ms interpulse interval). Independence was accepted when facilitation of the second  
213 pulse was  $\sim 10$  % or less. To assess basal synaptic transmission, stimulus input/fEPSP slope output  
214 curves were constructed over the range of 20 - 300  $\mu\text{A}$ . A minimum of four fEPSPs were averaged to  
215 yield a fEPSP slope measurement at each stimulus intensity. At the highest stimulus intensity (300  
216  $\mu\text{A}$ ), and where visible, the presynaptic fibre volley was measured as an indicator of the recruitment

217 of afferent axons. Paired-pulse facilitation (PPF), a commonly used index of the probability of  
218 neurotransmitter release, was assessed over an inter-stimulus interval of 50 – 350 ms, with the  
219 average of at least 2 fEPSPs yielding the slope measurement at each paired-pulse interval. In all  
220 experiments both pathways in each slice were tested for input-output and PPF profiles and all were  
221 taken into consideration in subsequent analyses.

222  
223 For the LTP and LTD experiments a stable baseline of at least 30 mins was achieved before theta-burst  
224 (TBS) or low-frequency stimulation (LFS) was delivered to one pathway. TBS consisted of bursts of 4  
225 stimuli at 100 Hz with 10 such bursts comprising a train. Each burst within a train was separated by  
226 200 ms. Trains were repeated 3 times with an inter-train interval of 20 seconds. LFS consisted of 900  
227 pulses at 1 Hz. The second pathway was not subject to TBS or LFS and served as a control for the  
228 stability of the recordings. Experiments were excluded from analysis if the control pathway  
229 deteriorated by more than 10 % within the 3 hours post-TBS, and 1 hour post-LFS, monitoring period.

230  
231 Given the deficit in basal synaptic transmission observed in MSK1 KD mice, care was taken to match  
232 the baseline strength of synaptic transmission, which involved adjusting the stimulus intensity to yield  
233 fEPSPs of ~3 mV across all groups. A one-way ANOVA showed no difference in baseline fEPSP  
234 amplitudes across the four experimental groups for either the LTP ( $F(3,28) = 0.676$ ;  $p = 0.574$ ) or LTD  
235 ( $F(3,22) = 0.975$ ;  $p = 0.422$ ) experiments. Electrophysiological recording parameters and the analysis  
236 of fEPSPs were under the control of WinLTP program (Anderson and Collingridge, 2007). LTD  
237 experiments, and the majority of LTP experiments, were performed in experimentally naïve mice of 3  
238 – 4 months of age. Experiments were interleaved and performed blind to the identity and housing

239 condition of the mice, which was revealed only after the experiments had been analysed and  
 240 genotype confirmed with post-hoc genotyping as required.

241

## 242 **Western Blotting**

243 Experimentally naïve mice (3-4 months of age) were killed by cervical dislocation as described above.  
 244 The brain was removed and individual hemispheres were snap-frozen in liquid nitrogen and stored at  
 245 -80°C. When required, samples were defrosted, the hippocampus dissected free and lysed in lysis  
 246 buffer containing: 50 mM Tris-HCl, pH 7.5, 1% Triton X-100, 0.1% SDS, 1 mM  $\text{Na}_3\text{VO}_4$ , 50 mM NaF, 5  
 247 mM  $\text{Na}_4\text{P}_2\text{O}_7$ , 0.27 M sucrose, 0.02%  $\text{NaN}_3$ , and protease inhibitor mixture tablets (Roche). The tissue  
 248 underwent mechanical disruption using a Dounce homogeniser. Samples were then stored on ice  
 249 before rotation at 4°C for 30 minutes, followed by centrifugation for 20 minutes and 12,000 g at 4°C.  
 250 The protein concentration of each sample was calculated using a standard BCA curve. Samples were  
 251 aliquoted, mixed with loading buffer, and stored at -20°C until required for western blotting.

252

253 After defrosting, samples were brought to 80°C for five minutes, spun briefly and the proteins were  
 254 separated using SDS-PAGE electrophoresis in an 8% gel. After separation, proteins were transferred  
 255 onto nitrocellulose blotting membrane (GE Healthcare) in a semi-wet system for 2.5 hours at 200  $\mu\text{A}$ .  
 256 The membrane was blocked in 10% Marvel milk powder and 0.5% TWEEN for one hour. Membranes  
 257 were incubated in GAPDH primary antibody (Table 1) in 1% milk powder 0.05% PBS TWEEN (PBS-T)  
 258 solution for two hours at room temperature, following which they were washed for 10 minutes four  
 259 times in 0.1% PBS-T. Samples were incubated overnight at 4°C with a second primary antibody (GluA1,  
 260 GluA2, EGR1, or Arc/Arg3.1; Table 1) then washed four times for 10 minutes in PBS-T. Membranes  
 261 were incubated for 1-2 hours in HRP-conjugated anti-rabbit antibody (1:10,000 dilution; ThermoFisher

262 #31460). After four 10-minute washes with PBS-T, membranes were incubated for two minutes in  
 263 Clarity Western ECL Substrate (BioRad) and imaged using the Image Quant LAS 4000 CCD  
 264 biomolecular imager. Image Studio Lite vs 5.2.5 was used to analyse the signal of the bands and the  
 265 protein of interest was normalised to GAPDH. Control blots confirmed that the GAPDH antibody gave  
 266 no detectable bands at the predicted molecular weights of GluA1, GluA2, EGR1 or Arc/Arg3.1.

267

268 Table 1 Antibodies used in the study

Primary antibody	Supplier (Cat #)	Concentration of primary Ab
EGR1	CST (4153S)	1:500
Arc/Arg3.1	Abcam (AB183183)	1:500
GluA1	Abcam (AB31232)	1:1000
GluA2	Abcam (AB 1768)	1:2000
GAPDH	CST (2118S)	1:160,000 (for Egr1 or Arc/Arg3.1 blots) 1:40,000 (for GluA1 or GluA2 blots)

269

## 270 Behavioural Procedures

271 Mice used were male aged 2.5 to 3.5 months and were scored for weight and against a battery of  
 272 tests for neurological signs (Wolf et al., 1996) before any behavioural experiment began. No  
 273 neurological signs were observed across any of the groups (data not shown). Different tests were  
 274 conducted at weekly intervals to avoid one test influencing another: the week commencing (w/c)  
 275 postnatal day (P) 70 open field and novel object; w/c P77 elevated plus maze; w/c P84 spontaneous  
 276 alternation; w/c P91- w/c 104 watermaze, a 2 week protocol.

277

## 278 *Open field and novel object*

279 These tests were run as two consecutive stages of the same experiment. Four open field boxes (Ugo  
280 Basile; 44 x 44 x 44 cm) were placed inside the empty water maze arena to form a square. Four mice  
281 were tested simultaneously. Each mouse was singly released in each box and tracked. Exposure to the  
282 open field lasted for 1 hour after which, for the novel object stage of testing, a 50 ml plastic vial  
283 (Falcon) was secured upside-down to the centre of the arena and the mouse was tracked for an  
284 additional hour.

285

#### 286 ***Elevated plus maze***

287 An 8-radial arm maze for mice (Ugo Basile) was placed within the empty water tank and raised 60 cm  
288 from the tank base. Four of the eight arms were kept open to form a plus shape; two of the arms had  
289 walls while the other two (opposite one another) were without walls. Each mouse was individually  
290 released in the centre of the maze and video tracked for 10 minutes.

291

#### 292 ***Spontaneous alternation for spatial working memory***

293 An 8-radial arm maze for mice (Ugo Basile) was placed within the circular confines of the tank used for  
294 the water maze. Four out the eight arms (with walls) were kept open to form a cross. The entrances  
295 to the other four arms were closed. Each mouse was individually released in the centre of the maze  
296 and video tracked for 10 minutes. The sequence of arm entries was scored. A correct alternation was  
297 considered when a mouse made no repetition in four entries (Mohler et al., 2007).

298

#### 299 ***Water maze for spatial reference memory***

300 Experimentally naïve mice were used and an inter-trial interval of 120 s over 4 daily trials was  
301 employed. The pool was filled daily with fresh water, which was made opaque by the use of UHT milk.

302

303 Stage 1 Habituation: each mouse was placed on a 20 cm diameter platform located in the centre of a  
 304 180 cm diameter pool filled with opaque water (28°C) and was allowed to observe the environment  
 305 for two minutes. The pool was surrounded by curtains which did not allow the distal visual cues to be  
 306 seen. Water level was ~1 cm above the top of the platform. Each mouse then received 3 consecutive  
 307 trials (different starting points) where it was left free to swim in the pool for a maximum of 2 minutes  
 308 and then placed on the platform and left there for 30 sec.

309 Stage 2 (days 2 and 3) Visual Cue: The platform was placed in the centre of the pool and a visible  
 310 object was placed upon it (yellow TV toy 6 x 6 x 5 cm). Each mouse received 4 consecutive trials  
 311 (different cardinal starting points) where it was left free to swim in the pool for a maximum of 2  
 312 minutes. Water level was ~ 1 cm above the platform surface. Water was kept at 26°C. The pool was  
 313 surrounded by curtains which did not allow the distal visual cues to be seen.

314 Stage 3 (days 4 to 7) Training: Curtains were removed. Water was kept at 26°C. The platform was  
 315 placed in the centre of the South-East or North-West quadrant and kept constant for any given  
 316 mouse. Water level was ~ 1 cm above the platform surface. Each mouse received 4 trials (different  
 317 starting points) where it was left free to swim in the pool for a maximum of 2 minutes and then left  
 318 on the platform for 30 sec.

319 Stage 4 (day 8) Precision Testing: The platform was reduced from 20 cm to 10 cm in diameter to test  
 320 for more specific memory of the location of the escape platform. All other parameters as per Stage 3.

321 Stage 5 (day 9) 24 hrs delay Probe trial: Water was kept at 26°C. The platform was removed and distal  
 322 spatial cues were present as per previous the stage. Each mouse received a single 120 sec trial.  
 323 Starting point was distal to the location of the platform during training; e.g. if platform was South-East  
 324 starting point was North.

325

326 ***Water maze reversal learning protocol for cognitive flexibility***

327 Stage 1 Habituation: As described above but two days were given instead of one.

328 Stage 2 (days 3 and 4) Visual Cue: As described above.

329 Stage 3 (days 5 to 7) Training: As described above.

330 Stage 4 (days 8 and 9) Reversal Learning: The platform was placed in the quadrant opposite to that  
331 used during Training. All other parameters as per Stage 3.332 Stage 5 (day 10) 24 hrs delay Probe trial: As described above. Starting point was distal to the location  
333 of the platform during Reversal Learning stage (Stage 4).334 Mice used for this experiment were experimentally naïve with respect to the water maze, but had  
335 undergone open field  $\pm$  novel object, the elevated plus maze and spontaneous alternation.

336

337 Behavioural tests were video-tracked and analysed using AnyMaze 4.99 video tracking system. All the  
338 behavioural experiments were conducted blind to genotype.

339

340 **RNA-Seq:**341 Hippocampal RNA was prepared from experimentally naïve mice of 3 – 4 months of age. Samples  
342 were prepared and analysed blind to the two genotypes and two housing conditions. The hippocampi  
343 from contemporaneous mice were used for western blotting for GluA1, GluA2, EGR1 and Arc/Arg3.1  
344 as described above.

345

346 ***RNA extraction and library preparation***

347 Hippocampi were extracted and then rapidly homogenized in Trizol (Invitrogen, #15596018). Total  
348 RNA was precipitated using isopropanol following the manufacturer's protocol and treated with  
349 DNaseI. RNA quality was checked using Nanodrop and a Qubit 4 fluorimeter (Invitrogen).

350

351 mRNA libraries were prepared using the TruSeqv2 (Illumina, #RS-122-2001) LS protocol in-house by  
352 the School of Life Sciences Genomics Facility. Briefly, poly-A mRNA was pulled down using poly-T  
353 magnetic beads, fragmented, and primed with random hexamers before first-strand synthesis.  
354 Following second-strand synthesis, blunt end repair was performed with a 3' to 5' exonuclease, and 3'  
355 ends adenylated. Adaptors were then ligated to the cDNA. All 24 library samples were quality checked  
356 on a 2100 bioanalyser (Agilent) and assayed on a Qubit 4 fluorimeter (Invitrogen) before being  
357 multiplexed 6 samples to a lane and sequenced at 150bp paired end on an Illumina HiSeq 4000. An  
358 average of 41.76M reads per sample were obtained (Extended Data Table 7-1 for Figure 7).

359

#### 360 ***Analysis Pipeline***

361 *Quality Control and Trimming:* Samples were de-multiplexed and the raw fastq files quality checked  
362 using FastQC (v0.11.3) (Andrews, 2010). Adaptor contamination was removed using Skewer (v0.2.2)  
363 (Jiang et al., 2014), with Illumina TruSeq v2 adapter lists, including reverse complements and  
364 theoretical PCR product. Fastq files were also trimmed if the mean quality of bases dropped below 10  
365 (4bp window) and only reads >50bp were kept. Adapter contamination removal was confirmed using  
366 FastQC. Paired fastq files for each sample (forward and reverse) were aligned to the mouse genome  
367 (GRCm38) using STAR aligner (v2.5) (Dobin et al., 2013), and annotated (GRCm38.87). Average read  
368 alignment was 92.29% after exclusion of the 2 samples that failed quality control (Extended Data  
369 Table 7-1 for Figure 7). Aligned BAM files were then loaded into IGV (v2.3.65) (Robinson et al., 2011)



370 and compared at the MSK1 gene locus, to check for a mismatch in the kinase domain of the MSK1  
371 gene introduced as a point mutation into the kinase dead mutants (Corrêa et al., 2012). QC metrics  
372 were calculated for each sample using SeqMonk (Andrews, 2018), examining high probe read counts  
373 across ribosomal RNA and mitochondrial genes, and observing how many reads fell within genes and  
374 exons. HtSeq (v0.6.1p1) (Anders et al., 2015) was then used to quantify read counts for individual  
375 genes, using default parameters, specifying unstranded reads and only unique read alignment. A  
376 minimum average PHRED quality score of 10 was necessary for reads to be counted.

377

378 Sample 34B contained a large percentage (~33%) of reads mapping to ribosomal RNA (rRNA).  
379 Additionally, sample 34B contained roughly 1% of the read number of sample 35A, indicating poor  
380 amplification of the cDNA library, and contained unacceptable sequence duplication levels. Ribosomal  
381 (rRNA) contamination (~25% of reads) was also observed in sample 33B. Both sample 34B and 33B  
382 were removed from further analysis based on poor quality control metrics.

383

384 Intra-group sample variation was observed to be quite high for some samples, and this interfered with  
385 obtaining good quality distinct expression. Therefore, several more samples were removed for each  
386 group, based on how well they correlated with other within-group samples. After alignment QC, and  
387 after removing samples displaying poor intra-group correlation, a new sample table was made for  
388 testing differential gene expression (Table 7-2). Differential gene expression lists for each condition  
389 comparison are included in Extended Data Table 7-3 for Figure 7.

390

391 Principal component analysis was conducted in R (3.5.0) (RTeam, 2018) using the DEseq2 package  
392 (v1.20.0) (Love et al., 2014). Differential gene expression statistical comparisons were conducted

393 using the Wald test statistic with a Benjamini-Hochberg-corrected p value cut-off of  $\leq 0.05$ , and a log2  
394 fold-change cut-off of 0.38 (corresponds to a 1.3-fold increase/decrease) used to define significant  
395 differentially expressed genes. Gene ontology enrichment analysis was performed using the topGO  
396 package (v2.32.0) (Alexa and Rahnenfuhrer, 2006) using the “classic” algorithm (Alexa and  
397 Rahnenfuhrer, 2006) and Fisher’s exact test for enrichment scoring against the ontology  
398 org.Mm.eg.db (v3.6.0) (Carlson, 2018). Multiple-testing correction was carried out using Benjamini-  
399 Hochberg correction, and GO-terms were considered significant at a corrected p-value  $\leq 0.01$ . Unless  
400 explicitly stated, default parameters were used for all tools and scripts used for these analyses and  
401 will be provided upon request.

402

#### 403 **Statistical Analysis**

404 Statistics were computed by IBM SPSS 25 using two-tailed one or two-way analysis of variance  
405 (ANOVA) with genotype and housing condition as the two between group factors and day of training,  
406 time-point or stimulus strength as within factor as appropriate, with simple main effects or main  
407 effects as the post-hoc comparison. In the absence of significant housing x genotype interactions,  
408 planned comparisons regarding the effects of genotype or housing were conducted, in keeping with  
409 the views expressed in expert treatments of statistics (Faraway, 2005; Kutner et al., 2005; Howell,  
410 2010; Wei et al., 2012; Laerd, 2017). The level of significance was taken to be  $p < 0.05$ . Data are  
411 reported as mean  $\pm$  SEM and bar graphs display individual data points.

412

413

414

415 **RESULTS**416 ***MSK1 is necessary for the full extent of experience-dependent enhancement of cognition***

417 To confirm that our enrichment protocol had tangible effects on animal behaviour, we initially  
 418 assessed the influence of enrichment on locomotor function and anxiety, the latter of which in  
 419 particular is sensitive to enrichment (Rogers et al., 2019). WT and MSK1 KD mice raised from birth in  
 420 standard housing behaved similarly when exposed to an open field arena (Figure 1A, B) and the  
 421 elevated plus maze (Figure 1C, D). In contrast to their counterparts raised in standard housing,  
 422 enriched animals of both genotypes displayed reduced locomotor activity in the open field and in  
 423 response to the introduction of a novel object (Figure 1A, B), and travelled further in the open arms of  
 424 the elevated plus maze, indicative of reduced anxiety (Figure 1C, D). These observations confirm the  
 425 effectiveness of the enrichment protocol in influencing behaviour, the absence of gross sensorimotor  
 426 impairments in the MSK1 KD mutant mice that could confound subsequent investigations, for  
 427 example, due to the high levels of MSK1 in the striatum and cerebellum (Heffron and Mandell, 2005),  
 428 and the ability of MSK1 KD mice to display some benefits of enrichment.

429

430 To assess hippocampus-dependent forms of learning and memory, we began by testing spatial  
 431 working memory using a spontaneous alternation task (Figure 2A). While standard housed mice of  
 432 both genotypes performed at comparable levels (Figure 2B), there was a significant effect of  
 433 enrichment that was reflected in significantly improved performance in WT mice, but not in the MSK1  
 434 KD animals (Figure 2B).

435

436 To extend these observations to spatial reference memory, the four groups of mice were tested in the  
 437 Morris water maze. Both MSK1 KD and WT mice benefitted from enrichment in learning the position

438 of the platform more rapidly than their standard housed counterparts, who performed comparably,  
439 as described previously (Daumas et al., 2017)(Figure 2C). Similarly, when the 20 cm platform was  
440 replaced with a 10 cm platform on Day 5 of training to test the accuracy of learning the location of the  
441 platform, enriched mice of both genotypes were better able to locate the smaller diameter platform  
442 than standard housed mice, with the more selective searching of the enriched mice obvious from heat  
443 maps of trajectories obtained on Day 5 (Figure 2D). These data suggest that MSK1 KD mice can display  
444 some cognitive benefits of enrichment. However, these benefits were not lasting over time: during  
445 the probe trial 24 hr later, enriched WT mice spent significantly more time in the training quadrant  
446 than their standard housed counterparts, while no enrichment-induced improvement was observed  
447 in the MSK1 KD mice (Figure 2E, F). MSK1 is therefore required for the full extent of enrichment-  
448 induced persistence of spatial reference memory.

449  
450 To further probe the requirement for MSK1 in the cognition-enhancing effects of enrichment, new  
451 experiments with four additional groups of water maze-naïve animals were conducted using the  
452 Lipp/Wolfer protocol for cognitive flexibility (Lipp and Wolfer, 1998). After training to asymptotic  
453 levels by day 3, the location of the escape platform was moved to the opposite quadrant and  
454 performance assessed on days 4 (R1) and 5 (R2) (Figure 3A). As expected, switching the location of the  
455 platform to the opposite quadrant resulted in longer escape latencies across all groups on the first  
456 exposure on reversal day 1 (Figure 3A, R1). However, by the second day of platform reversal (R2), the  
457 enriched WT mice escaped more quickly than standard housed WT mice. This pattern was not seen in  
458 enriched MSK1 KD mice, with latency to platform and heat maps of their swimming trajectory  
459 observed to be comparable to that of standard housed WT mice (Figure 3A, B). This suggests a  
460 requirement for MSK1 in the cognitive flexibility required for reversal learning.

461

462 Moreover, the failure to retain information over 24 hours was again shown by MSK1 mutant mice, but  
 463 now concerning the new location for escape: in the probe trial given 24 hrs later (Figure 3C, D), both  
 464 standard housed and enriched WT mice showed clear preference to navigate to the most recent  
 465 (reversed) location of the platform. In contrast, MSK1 mutant mice either displayed no preference  
 466 between the new and the old escape locations or, in the case of the enriched MSK1 KD mice, even a  
 467 preference for the former location of the platform used for training on days 1 – 3 (Figure 3C, D).

468

469 MSK1 KD mice that had experienced enrichment seemed to fare worse on both probe trial tests for  
 470 memory persistence. To examine this in greater detail we considered the amount of time spent in the  
 471 appropriate quadrant for both the small platform experiment (Figure 2C-F; target quadrant) and the  
 472 reversal learning experiment (Fig 3A-D; reversal quadrant). To compensate for differences in  
 473 performance across these two trials, we normalised performance across all groups to the mean of the  
 474 percentage time spent in the appropriate quadrant by standard housed WT mice. Bringing these two  
 475 data sets together in this way increases the power of the observations and allows more robust  
 476 conclusions to be drawn as to the performance of enriched MSK1 KD mice. This analysis (Figure 3E)  
 477 showed a significant genotype x housing interaction with enhanced memory ability in enriched WT  
 478 mice compared to enriched MSK1 KD mice. These observations indicate that enrichment improves  
 479 both spatial working and reference memory in WT mice, and that both the persistence of memory  
 480 and reversal learning, an index of cognitive flexibility, requires the kinase activity of MSK1.

481

482 ***Environmental enrichment induces synaptic homeostasis in an MSK1-dependent manner***

483 To establish the extent to which these cognitive impairments were reflected at the cellular level, we  
484 performed electrophysiological recordings of basal excitatory synaptic transmission from area CA1  
485 (Figure 4). We have previously reported an enhancement in mEPSC amplitude after enrichment in  
486 WT, but not MSK1 KD mice (Corrêa et al., 2012; Lalo et al., 2018). We also reported that mEPSCs in  
487 MSK1 KD mice were ~10% smaller than those recorded from WT mice (Corrêa et al., 2012; Lalo et al.,  
488 2018), and more recently showed that this translated into smaller evoked field excitatory potentials  
489 (fEPSP) in area CA1 of MSK1 KD hippocampal slices (Daumas et al., 2017). Thus, in order to establish  
490 whether i) environmental enrichment also enhanced synaptic transmission at the population fEPSP  
491 level in WT animals and ii) whether the basal fEPSP deficit in synaptic transmission in MSK1 KD mice  
492 persisted or was ameliorated after enrichment, we constructed input/output curves of stimulation  
493 strength vs. the slope of the fEPSP in slices taken from animals raised under standard housing or  
494 environmentally-enriched housing (Figure 4A).

495

496 Consistent with previous observations under standard housing conditions (Daumas et al., 2017), basal  
497 synaptic transmission in MSK1 KD slices was substantially reduced compared to that observed in WT  
498 slices (Figure 4A). This was not due to an impairment in the recruitment of presynaptic axons as the  
499 fibre volley amplitudes (Figure 4A) did not differ between these two groups, nor indeed between the  
500 groups having undergone enrichment. To establish whether the reduced synaptic transmission  
501 reflected differences in the expression of glutamate AMPA receptors, which are responsible for the  
502 majority of excitatory synaptic transmission at CA1 synapses, we conducted western blots for GluA1  
503 and GluA2, the two primary AMPAR subunits contributing to synaptic transmission in area CA1  
504 (Tsuzuki et al., 2001; Lu et al., 2009; Renner et al., 2017; Terashima et al., 2017; Diering and Huganir,

2018). No differences in GluA1 or GluA2 expression were observed under standard housing conditions that could explain the deficit in synaptic transmission observed in MSK1 KD mice (Figure 4B).

The deficit in basal synaptic transmission in MSK1 KD mutant mice compared to WT mice persisted in animals raised in enriched conditions (Figure 4A). Moreover, enrichment had no discernible effect on basal synaptic transmission in the MSK1 KD mutants; the input-output curves of standard housed and enriched MSK1 KD mice essentially overlapped. In contrast, synaptic transmission in slices from enriched WT mice was weaker over the entire range of stimulus strengths, but did not reach statistical significance. While enrichment had no effect on GluA1 expression (Figure 4B), there was an enrichment x genotype interaction for GluA2 levels such that there was an apparent increase and decrease in WT and MSK1 KD mice, respectively. These changes in GluA2 expression do not directly translate to the observed effects of enrichment on synaptic transmission, which saw decreases and no change in WT and MSK1 KD mice, respectively, but suggest that both experience and MSK1 can influence the expression of glutamate AMPA receptor subunits.

One potential explanation for the observation of a tendency to reduced synaptic transmission in WT mice is that there has been a reduction in the probability of evoked glutamate release, such that on average there are fewer synapses and postsynaptic AMPA receptors activated per stimulus. To test this we constructed paired-pulse facilitation (PPF) profiles over the inter-pulse interval of 50 – 350 ms (Figure 4C) as an index of the initial probability of glutamate release (Jackman and Regehr, 2017). Under standard housing conditions, and as reported previously (Daumas et al., 2017), the PPF profile of MSK1 KD mutant and WT slices were not different from one another. This suggests that MSK1 does not play a role in regulating transmitter release under standard housing conditions.

528

529 Similar to the lack of effect on basal synaptic transmission (Figure 4A), raising MSK1 KD mice in  
 530 enriched conditions had no effect on the PPF profile compared to their standard housed counterparts.  
 531 However, WT mice raised in enriched housing showed a clear, consistent and significant *enhancement*  
 532 of PPF across the entire PPF range (Figure 4C). Since PPF is inversely proportional to the initial  
 533 probability of neurotransmitter release, this enhancement of PPF in WT slices likely reflects a  
 534 reduction in the probability of glutamate release, and may explain the consistent decrease in fEPSP  
 535 strength observed in enriched WT mice (Figure 4A). As such, these observations of changes in the  
 536 probability of release in WT, but not MSK1 mutant hippocampal slices may be an adaptive, MSK1-  
 537 dependent homeostatic response to the increase in miniature excitatory postsynaptic currents we  
 538 previously reported in enriched WT mice (Corrêa et al., 2012), potentially to limit network excitability.

539

540 An alternative explanation, that the differences in synaptic transmission between genotypes and after  
 541 enrichment in WT mice reflect corresponding decreases in the number of dendritic spines, are not  
 542 supported by spine density measurements made from apical dendrites in Golgi-impregnated CA1  
 543 neurons (Figure 5). As reported previously (Corrêa et al., 2012), CA1 apical dendrite spine density was  
 544 significantly higher in MSK1 KD mice than in WT mice (Figure 5A, B), with a trend towards greater  
 545 spine density after enrichment in both genotypes.

546

#### 547 ***The experience-dependent enhancement of the dynamic range of synapses requires MSK1***

548 These measures of basal synaptic transmission, the probability of glutamate release, GluA subunit  
 549 expression and spine density point to subtle cellular effects of enrichment that would not be expected  
 550 to contribute appreciably to either the observed enhancement of cognition in WT mice, or the



551 inability of MSK1 KD mice to display the full cognitive benefits of enrichment. Accordingly, to probe  
552 the potential cellular basis of the enrichment- and MSK1-dependent enhancement of spatial learning  
553 and memory, we performed electrophysiological recordings of synaptic plasticity in area CA1 of  
554 hippocampal slices prepared from standard housed and enriched WT and MSK1 KD mutant mice.

555

556 Since there is widespread agreement that activity-dependent changes in the efficacy of synaptic  
557 transmission, which have been observed after enrichment (Ohline and Abraham, 2019), underlie the  
558 ability of animals to learn and remember (Takeuchi et al., 2014), we predicted that: a) enrichment  
559 would enhance the ability of synapses to display activity-dependent modifications of synaptic  
560 strength, and b), given the impairment of cognition observed in MSK1 mutant mice, any enrichment-  
561 induced synaptic enhancement would require MSK1, and hence be absent in the MSK1 mutants. We  
562 thus performed dual-pathway long-term potentiation (LTP) and long-term depression (LTD)  
563 experiments from area CA1 in hippocampal slices from standard housed and enriched WT and MSK1  
564 KD mice, where one pathway served as a time control, and the other pathway was subjected to  
565 plasticity-inducing stimulation. Consistent with our hypotheses, both LTP (Figure 6A) and LTD (Figure  
566 6B) were significantly enhanced in the CA1 region of hippocampal slices prepared from WT mice that  
567 had received enrichment. In stark contrast, neither LTP nor LTD were affected by enrichment in the  
568 MSK1 mutant mice, where the extent of synaptic plasticity was comparable to that obtained from  
569 standard housed mice of both genotypes (Figure 6A, B).

570

571 These observations indicate that MSK1 is required for the bidirectional expression of the enhanced  
572 plasticity associated with environmental enrichment, i.e. an expansion of the dynamic range of  
573 synapses, which occurred in the absence of appreciable changes in basal synaptic transmission or

574 dendritic spine density (Figures 4 and 5), and in experiments where baseline fEPSPs were carefully  
 575 stimulus-matched for amplitude to take into account differences in basal synaptic transmission  
 576 between MSK1 KD mutants and WT mice (see Materials and Methods: *Hippocampal slice preparation*  
 577 *and extracellular recordings*).

578  
 579 To quantify the extent of the enrichment- and MSK1-dependent expansion of the synaptic dynamic  
 580 range, we compared, from the weakest to the strongest, the range of synaptic strengths recorded in  
 581 the LTD and LTP experiments. On this basis we calculated a ~28% increase in the synaptic dynamic  
 582 range in WT animals, but essentially no change in synaptic strength in response to enrichment in  
 583 MSK1 KD mice (~5 %). The bidirectional enhancement of synaptic strength in enriched WT animals  
 584 can be appreciated in a plot of the cumulative distribution of individual LTD and LTP values for each of  
 585 the experiments in each of the four groups of animals (Figure 6C). A complementary comparison  
 586 examined the net change from baseline (100 %) in each of the LTP and LTD experiments (Figure 6D)  
 587 across both genotype and housing conditions. This analysis revealed that enrichment selectively  
 588 enhanced the dynamic range of synapses in WT mice, and thus demonstrates that the kinase activity  
 589 of MSK1 is necessary for the experience-dependent bidirectional expansion of synaptic strength.

#### 591 ***Experience influences gene expression in an MSK1-dependent manner***

592 Given the dependence of persistent changes in synaptic function and cognition on gene expression  
 593 (Alberini and Kandel, 2014), and the importance of MSK1 in regulating transcription (Reyskens and  
 594 Arthur, 2016), including for key plasticity-related proteins such as Arc/Arg3.1 (Hunter et al., 2017), we  
 595 addressed the molecular mechanisms downstream of MSK1 by examining patterns of gene expression  
 596 in WT mice in which MSK1 was active, and in kinase-dead animals in which it was not. RNA-Seq was

597 performed on hippocampal tissue obtained from the four groups of mice. A principal component  
 598 analysis (Figure 7A, B) revealed that a striking 73% of the variance in gene expression was captured by  
 599 the first principal component, corresponding to housing type, with both WT and MSK1 KD standard  
 600 housed mice clustering together (Figure 7B). The enriched WT group was readily distinguishable from  
 601 these two groups, with members clustered tightly together. In contrast, the enriched MSK1 KD mice  
 602 were distinct from both standard housed mice of both genotypes and enriched WT mice (Figure 7B).  
 603 Thus, enrichment had an effect on gene expression in MSK1 KD mice, but it appeared to be an  
 604 uncoordinated or random response to enrichment, in stark contrast to the tightly regulated  
 605 transcriptomic response in WT mice.

606  
 607 Differential gene expression analysis revealed that, while there were only three differentially-  
 608 expressed genes between standard housed WT and MSK1 KD mice (Figure 7C, Extended Data Tables  
 609 7-3, 7-4 for Figure 7), enrichment affected the regulation of 261 unique genes in WT mice (Figure 7C,  
 610 D; Extended Data Table 7-5 for Figure 7). In contrast, only 42 genes showed selective regulation by  
 611 enrichment in the MSK1 KD mice (Figure 7C, D; Extended Data Table 7-6 for Figure 7), with an  
 612 additional 214 genes regulated by enrichment in both WT and MSK1 KD mice (Figure 7C, D; Extended  
 613 Data Table 7-7 for Figure 7). These data indicate that the majority of genes affected by enrichment  
 614 are regulated by MSK1.

615  
 616 To identify the function of the genes regulated by enrichment, we performed Gene Ontology (GO)  
 617 analysis on genes differentially expressed in response to enrichment (Figure 7D, E, F). The 214 MSK1-  
 618 independent genes were distributed amongst 153 GO categories (Figure 7E; Extended Data Table 7-8  
 619 for Figure 7), which included cell adhesion, extracellular matrix and structure organisation and,

notably, the regulation of MAPK signalling, of which MSK1 is an integral part. A similar GO analysis of the 261 unique MSK1-dependent genes revealed a restricted distribution amongst only 10 GO categories (Figure 7F; Extended Data Table 7-9 for Figure 7), with 7 of these being unique to the MSK1-dependent genes. These unique categories encompassed microtubule bundle formation, cilium organization, assembly and movement, as well as ciliary axoneme assembly and collagen fibril organisation.

***MSK1 is necessary for an experience-dependent homeostatic downregulation of plasticity gene expression***

An examination of specific genes regulated by both MSK1 and enrichment revealed 11 genes (Figure 8A, B; Extended Data Table 8-10 for Figure 8) that were significantly differently expressed between enriched WT and MSK1 KD mutant mice. In particular, two genes regulated by neurotrophins and the MAPK cascade were strongly downregulated in enriched WT mice: Sprouty4 (Spry4) (Cabrita and Christofori, 2008) and early growth response protein 1 (Egr1/Zif268/NGFI-A) (Veyrac et al., 2014; Duclot and Kabbaj, 2017). This unexpected downregulation of Egr1, which has repeatedly been shown to be elevated acutely in response to enrichment, plasticity- and learning and memory-inducing stimuli (Pinaud, 2004; Veyrac et al., 2014; Duclot and Kabbaj, 2017), prompted a curated investigation of genes relevant to the activation of MSK1. This analysis (Figure 9A) revealed a striking and unexpected enrichment-induced downregulation of the MSK1 signalling cascade, including of MSK1 itself (Figure 9B), but not of the related MSK2 isoform (Figure 9C). In addition to downregulation of Egr1, downregulation was also observed of the MSK1 substrate CREB, and of the key plasticity-related protein Arc/Arg3.1 (Epstein and Finkbeiner, 2018), which we have previously shown was regulated in an MSK1-dependent manner during both homeostatic synaptic plasticity (Corrêa et al., 2012) and in

643 response to BDNF (Hunter et al., 2017). That this enrichment-induced reduction of MSK1 gene  
644 expression had tangible effects on Egr1 and Arc/Arg3.1 protein expression was confirmed by western  
645 blots from contemporaneous hippocampal tissue, which showed strong enrichment-induced  
646 downregulation of Egr1 and Arc/Arg3.1 protein levels exclusively in WT mice (Figure 9D, E). These  
647 data indicate that MSK1 orchestrates an experience-dependent homeostatic downregulation of key  
648 plasticity-related proteins.

649

650 **DISCUSSION**

651 The molecular pathway that links the external environment to the genomic response that underpins  
652 experience-dependent neuronal and cognitive adaptations has remained elusive, but is important to  
653 identify given the potential for pharmacological manipulation to remediate the cognitive deficits  
654 associated with congenital, acquired and age-related cognitive impairment (Guerrieri et al., 2017;  
655 Consorti et al., 2019). While both BDNF and a range of plasticity-related proteins have been  
656 implicated in the neuronal response to enrichment (Cowansage et al., 2010; Sale et al., 2014; Rogers  
657 et al., 2019), the mechanism that allows experience to couple BDNF to plasticity-related proteins has  
658 yet to be described.

659

660 ***MSK1 orchestrates an experience-dependent genomic homeostasis***

661 We have shown previously that mice lacking the kinase activity of MSK1, an enzyme regulated by  
662 BDNF (Arthur et al., 2004; Daumas et al., 2017; Hunter et al., 2017), fail to upregulate miniature  
663 excitatory synaptic currents in response to either enrichment in vivo, or activity deprivation in vitro  
664 (Corrêa et al., 2012; Lalo et al., 2018). This selective mutation of the MSK1 gene obviates concerns  
665 regarding a structural role for MSK1 (Gutierrez-Mecinas et al., 2011) that may contribute to the basal  
666 (Chwang et al., 2007; Choi et al., 2012; Karelina et al., 2012) and enrichment-induced (Karelina et al.,  
667 2012) deficits in learning and memory and neurogenesis observed in mice in which MSK1 has been  
668 constitutively deleted. Instead, the MSK1 kinase dead (KD) mutation allows the influence of  
669 experience to be examined against an intact basal synaptic plasticity and cognitive repertoire  
670 (Daumas et al., 2017).

671

672 Using mice lacking the kinase activity of MSK1, we have presented evidence from several  
673 independent, but convergent lines of investigation, from genes to behaviour, that MSK1 is a strong  
674 candidate for acting as an important link between the environment and the genome. While we have  
675 used male mice, which predominate in environmental enrichment research (Simpson and Kelly, 2011;  
676 Girbovan and Plamondon, 2013), it is unlikely that our observations would not generalise to females  
677 given the reported increases in BDNF production and MAPK activity in female rodents after  
678 enrichment (Bakos et al., 2009; Ramirez-Rodriguez et al., 2014). However, this should be empirically  
679 tested in subsequent studies.

680

681 Through comparing WT and MSK1 KD mice, we have shown that the kinase activity of MSK1 has an  
682 obligatory role in the regulation of the transcriptional response to experience; the modulation of the  
683 dynamic range of synapses; the persistence of memory, and cognitive flexibility. We propose that  
684 these effects are causally linked. A coordinated and MSK1-dependent pattern of gene expression  
685 likely facilitates the growth and development of the enriched brain, with Sprouty4 and genes  
686 regulating the primary cilium being especially targeted. Sprouty4 exerts an inhibitory influence on the  
687 actions of neurotrophins (Cabrita and Christofori, 2008; Alsina et al., 2012) and on axonal outgrowth  
688 (Hausott et al., 2012). The experience-dependent downregulation of Sprouty4 would thus be  
689 expected to remove this inhibition allowing greater influence of neurotrophins on neuronal structure  
690 and function. The primary cilium is a cellular organelle that protrudes from the surface of virtually all  
691 mammalian cells, including neurons, and has important signalling properties (Nachury and Mick,  
692 2019). The cilium plays an important role in dendritic arborisation (Guadiana et al., 2013), neuronal  
693 development and neurogenesis (Gomez-Gamboa et al., 2014; Valente et al., 2014; Lepanto et al.,  
694 2016), and has been implicated in the maturation of neuronal circuits, synaptic plasticity and learning

695 and memory (Kumamoto et al., 2012; Rhee et al., 2016). These, and other, MSK1-dependent genes  
 696 are therefore likely to contribute to the frequently observed changes in brain structure and function  
 697 that support experience-dependent enhanced cognition (Rosenzweig and Leiman, 1968; Rosenzweig  
 698 and Bennett, 1996; Kolb and Whishaw, 1998). Moreover, once having facilitated the functional and  
 699 structural response to enrichment, MSK1 orchestrates a genomic homeostatic scaling characterised  
 700 by the downregulation of the transcription factor EGR1 and the plasticity-related protein Arc/Arg3.1.  
 701 The function of this unexpected downregulation, which is also observed in upstream MSK1-activating  
 702 kinases such as ERK2 and P38, and indeed of MSK1 itself, may be to stabilise and preserve the  
 703 neuronal networks, synaptic plasticity and cognitive enhancement arising from experience. Thus, the  
 704 normal pattern of MSK1-dependent gene expression can be homeostatically down-tuned in response  
 705 to enrichment in WT mice, but this adaptive ability is lost in the MSK1 KD mice, likely resulting in the  
 706 genomic, synaptic and cognitive impairments seen in the mutants.

707

708 ***MSK1 regulates the dynamic range of synapses and is required for the full expression of experience-***  
 709 ***dependent enhancement of cognition.***

710 In parallel, experience augments the dynamic range of hippocampal synapses in an MSK1-dependent  
 711 manner through the enhancement of both LTP and LTD. MSK1 thus enables synapses to both store  
 712 more information, and potentially be more responsive to prevailing synaptic and neuronal activity.  
 713 This may manifest as the ability to rapidly switch from one learned behaviour, potentially by both  
 714 weakening established neuronal circuits in an LTD-like manner, and mastering another, and for  
 715 longer, through LTP-like strengthening of new networks. Hippocampus-dependent behavioural  
 716 correlates of these forms of synaptic plasticity, reversal learning and the persistence of spatial  
 717 memory, were both impaired in mice lacking the kinase activity of MSK1. This suggests that the MSK1-



718 dependent expansion of the dynamic range of synapses increases the information capacity of  
 719 synapses, underpins the experience-dependent enhancement of cognition and thus provides a  
 720 plausible mechanism for the consistent improvements in cognition repeatedly observed in response  
 721 to enrichment since their first description in the 1940s by Donald Hebb (Hebb, 1947, 1949). Equally,  
 722 the increased number of dendritic spines in MSK1 KD mice observed here and in a previous study  
 723 (Correa et al., 2012) suggest that MSK1 influences spine density, either constitutively as a regulator of  
 724 gene expression, or in an activity-dependent manner in response to synaptic activity. This  
 725 dysregulation of spine number in the MSK1 KD mutant may contribute in particular to the cognitive  
 726 impairments seen in MSK1 KD mice after enrichment, and has parallels with the greater spine density  
 727 and impaired cognition observed in both human autism spectrum disorder and animal models of  
 728 autism (Coley and Gao, 2018; Nakai et al., 2018). MSK1 may thus coordinate the neuronal  
 729 mechanisms and networks supporting synaptic structure, function, plasticity and cognition through  
 730 the regulation of gene expression. After enrichment, the MSK1-driven genomic downregulation of  
 731 plasticity-related proteins leads to a reduced baseline against which activity-dependent elevations in  
 732 their transcription may proportionally have greater synaptic and cognitive impact.

733

734 ***MSK1 is a key regulator of experience- and activity-dependent genomic, synaptic and cognitive***  
 735 ***plasticity.***

736 The recruitment of MSK1 during exposure to a complex environment underpins a range of adaptive  
 737 genomic, molecular and synaptic responses that contribute appreciably to the experience-dependent  
 738 enhancement of cognition. Moreover, by initiating the downregulation of key plasticity-related genes,  
 739 MSK1 plays a pivotal role in ensuring the stability of the new and improved experience-dependent  
 740 genomic, neuronal and cognitive landscape, and one that is potentially primed to respond more

741 effectively to new challenges. MSK1, from regulating homeostatic synaptic scaling in vitro (Corrêa et  
742 al., 2012) to initiating genomic homeostasis in response to experience in vivo, thus represents a key  
743 molecular sensor linking the environment and prevailing synaptic activity to the genome and the  
744 ensuing adaptive neuronal and cognitive response.

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746

747 **Author contributions**

748 LP performed and analysed the electrophysiological studies and prepared RNA; LM performed and  
749 analysed the behavioural studies, prepared Golgi sections of brain tissue and supervised spine  
750 analysis; DDC performed the RNA-Seq analysis; PR conducted the western blots; MT analysed spine  
751 density; DH guided the RNA-Seq analysis; JSCA provided the MSK1 KD mice and expertise on  
752 molecular analyses; BGF obtained funding, conceived and oversaw the study. LP, LM, DDC, PR, DH,  
753 JSCA, BGF contributed to the writing of the manuscript. All authors approved the final version of the  
754 manuscript.

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935

936 **FIGURE LEGENDS**

937 **Figure 1. Environmental enrichment influences exploration and anxiety independently of MSK1.**

938 **A** Mice (n = 16 per group) of both genotypes (WT and MSK1 KD) and housing condition (standard  
 939 housed (SH) and enriched (EE)) were placed in an open field for 60 mins (left half of graph) after which  
 940 a 50 ml plastic tube was introduced into the arena (right half of graph; novel object). Animal  
 941 locomotion was tracked for the entire period. During the first 60 minutes a RM-ANOVA showed an  
 942 effect of time for all groups ( $F(5,300) = 149.89$ ,  $p = 0.001$ ) indicating a reduction in activity over this  
 943 period, and also a housing effect ( $F(1,60) = 12.10$ ,  $p = 0.001$ ) indicating that standard housed mice  
 944 were more active regardless of their genotype. During the second 60 minutes of the test (after the  
 945 introduction of the novel object) a RM-ANOVA showed an effect of time for all groups ( $F(5,300) =$   
 946  $220.46$ ,  $p = 0.001$ ) indicating a reduction in activity over time, and also a housing effect ( $F(1,60) =$   
 947  $10.31$ ,  $p = 0.002$ ) showing that standard housed mice remained more active in response to the  
 948 introduction of a novel object regardless of their genotype. Data are presented as mean  $\pm$  SEM. Data  
 949 points have been offset for clarity. **B** Heat maps of activity before and after introduction of the novel  
 950 object for each of the four groups of animals. **C** Mice (n = 16 per group) were placed on the central  
 951 area of an elevated plus maze and allowed to explore for 10 mins during which time their activity was  
 952 video tracked. Enriched mice of either genotype travelled further in the open arms compared to mice  
 953 housed under standard conditions ( $F(1,60) = 11.21$ ,  $p = 0.001$ ). Bars show the mean  $\pm$  SEM and  
 954 individual data points. **D** Occupancy heat maps for each of the groups, with the closed arms of the  
 955 elevated plus maze running vertically.

956 **Figure 2. The kinase activity of MSK1 is necessary for enrichment-induced memory persistence**

957 **A** In a spontaneous alternation test of spatial working memory, mice (n = 15 to 16 per group) were  
 958 exposed for 10 mins to a radial maze (with walls) in which four arms were open to the mice. The mice

959 scored correctly if four different arms were visited before a return visit occurred to any one of the  
960 arms. **B** The percentage of correct alternations was higher for enriched mice (EE) regardless of  
961 genotype (housing effect:  $F(1,58) = 8.15$ ;  $p = 0.006$ ). While there was no significant genotype x  
962 housing interaction, the difference between enrichment and standard housed (SH) mice was greater  
963 in the WT, which showed a significant improvement ( $F(1,58) = 6.41$ ;  $p = 0.014$ ), compared to the  
964 MSK1 KD (KD) that showed no improvement ( $F(1,58) = 2.22$ ;  $p = 0.14$ ). Bar graph depicts mean  $\pm$  SEM  
965 and individual data points. **C** Mice ( $n = 10$  to  $14$  per group) were trained on the water maze over five  
966 consecutive days. All groups showed learning of the platform location over the first stage of training  
967 (4 days; D1-D4) with a 20 cm platform. A RM-ANOVA, with genotype and housing as factors showed a  
968 significant effect of session ( $F(3,129) = 36.16$ ;  $p = 0.000$ ) on the latency to reach the escape platform.  
969 It also showed a main effect of housing ( $F(1,43) = 4.94$ ;  $p = 0.032$ ) where enriched mice of both  
970 genotypes performed better than standard housed mice, with no difference in performance between  
971 standard housed mice. On the second stage of the training (1 day; D5), in which a 10 cm platform was  
972 employed to test for more precise spatial reference memory, the ANOVA on the latency to reach the  
973 escape platform with between factors as genotype and housing again showed an effect of housing  
974 with better performance in enriched mice ( $F(1,43) = 8.45$ ;  $p = 0.006$ ). Data shown as mean  $\pm$  SEM.  
975 Data points are offset for clarity. **D** Heat maps of trajectories for all four groups. The platform was  
976 located in the south east quadrant. More selective searching (greater occupancy of the training  
977 quadrant) can be seen in the enriched mice. **E** On the probe trial 24 hours after D5, enriched WT mice  
978 showed the best retention for the spatial location of the escape platform, whereas the enriched MSK1  
979 KD mice showed the worst performance. The ANOVA on the total time spent on the target quadrant  
980 (where the platform had been previously) showed a significant interaction between genotype and  
981 housing ( $F(1,43) = 6.65$ ;  $p = 0.013$ ) and the simple main effect showed a significant difference

982 between enriched WT mice and enriched MSK1 KD mice ( $F(1,43) = 4.84$ ;  $p = 0.033$ ). Bar graph depicts  
 983 mean  $\pm$  SEM and individual data points. **F** The retention deficit in the enriched MSK1 KD mice was  
 984 observed across the entire probe trial duration where they spent consistently less time in the  
 985 appropriate quadrant compared to other groups. Data shown as mean  $\pm$  SEM. Data points are offset  
 986 for clarity.

987 **Figure 3. The kinase activity of MSK1 is necessary for enrichment-dependent cognitive flexibility**  
 988 **and long-term memory**

989 **A** All groups ( $n = 12$  or  $13$  per group) showed learning over the first stage of training on the Morris  
 990 water maze (3 days; D1 – D3). A RM-ANOVA with genotype and housing as between factors showed a  
 991 significant effect of session ( $F(2,92) = 71.91$ ;  $p = 0.000$ ) on the latency to reach the escape platform. It  
 992 also showed a main effect of housing ( $F(1,46) = 7.76$ ;  $p = 0.008$ ) where the enriched mice performed  
 993 better than standard housed mice. On the reversal learning stage (2 days; R1 – R2) a RM-ANOVA on  
 994 the latency to reach the new escape platform location showed an effect of session ( $F(1,46) = 16.15$ ;  $p$   
 995  $= 0.000$ ), genotype ( $F(1,46) = 15.66$ ;  $p = 0.000$ ) and housing ( $F(1,46) = 6.59$ ;  $p = 0.014$ ) indicating that  
 996 all groups learned over the two days, but WT mice performed better than MSK1 KD mutant mice, and  
 997 enriched mice better than standard housed mice. There was no significant interaction between  
 998 genotype and housing, but on the first day of reversal learning (R1) there was a significant difference  
 999 between standard housed WT mice and standard housed MSK1 KD mice ( $F(1,46) = 5.54$ ;  $p = 0.023$ ).  
 1000 On R2 there was a significant difference between enriched WT mice and enriched MSK1 KD mice  
 1001 ( $F(1,46) = 6.42$ ;  $p = 0.015$ ) and again between standard housed WT mice and standard housed MSK1  
 1002 KD mice ( $F(1,46) = 6.32$ ;  $p = 0.016$ ). Data shown as mean  $\pm$  SEM. Data points are offset for clarity. **B**  
 1003 Occupancy plots for the second day of reversal learning (R2) which show preferential searching in the  
 1004 reversal quadrant (south east) by enriched WT mice. **C** On the probe trial 24 hours later, enriched WT

mice showed the best goal-directed behaviour for the reverse location of the escape platform, while the enriched MSK1 KD mice again showed the worst performance. While there was no significant interaction between genotype and housing, an ANOVA on the latency to enter the reversal location of the platform showed a significant effect for genotype ( $F(1,46) = 7.51$ ;  $p = 0.009$ ) and also a significant difference between enriched WT and enriched MSK1 KD mice ( $F(1,46) = 6.39$ ;  $p = 0.015$ ). Bar graph depicts mean  $\pm$  SEM and individual data points. **D** Inset are heat maps of occupancy in the water maze during the 120 s probe trial for the four groups. Enriched WT mice show activity concentrated in the reversal learning quadrant (south east). **E** A comparison of 24 hr probe trial performance across the two experiments (small platform, Figure 2E, F and reversal learning, Figure 3C, D), where performance across groups was normalised to that of WT standard housed mice, showed a significant genotype  $\times$  housing interaction ( $F(1,93) = 8.49$ ,  $p = 0.004$ ), where enriched MSK1 KD mice performed significantly worse than their enriched WT counterparts ( $F(1,93) = 11.48$ ,  $p = 0.001$ ) who in turn performed better than standard housed WT mice ( $F(1,93) = 5.08$ ,  $p = 0.027$ ). Data from 23-27 mice per group. Broken line indicates level of performance when normalised to the respective WTSH mean.

**Figure 4. Enrichment has minimal effects on basal synaptic transmission, but differentially affects GluA2 expression and reduces the probability of neurotransmitter release in an MSK1-dependent manner.**

**A** MSK1 KD mice raised in standard (KD SH) and enriched (KD EE) housing displayed reduced synaptic transmission compared to WT mice (WT SH; WT EE), as previously described for standard housed WT and MSK1 KD mice (Daumas et al., 2017). A RM-ANOVA showed a significant effect of stimulus strength ( $F(5,560) = 632.11$ ;  $p = 0.000$ ) and a significant effect of genotype ( $F(1,112) = 43.99$ ;  $p = 0.000$ ), where the fEPSPs were larger in the WT mice regardless of their housing condition. Insets show representative fEPSPs at increasing stimulus strengths for each genotype and housing condition.



Data are presented as mean  $\pm$  SEM. Data points have been offset for clarity. **B** Western blots of GluA1 (left; open arrow) and GluA2 (right; grey arrow) expression showing two unique samples per blot from each of the four experimental groups. Quantification relative to GAPDH expression (filled arrow on blots) showed a significant genotype  $\times$  housing interaction for GluA2 ( $F(1,12) = 5.71$ ;  $p = 0.034$ ). One of two blots for each is shown. WT enriched, WE; WT standard housed, WS; MSK1 KD enriched, KE; MSK1 KD standard housed, KS. The ladder blot was digitally superimposed on the blots for GluA1, GluA2 and GAPDH. **C** There was no difference in paired-pulse facilitation (PPF) between standard housed WT and MSK1 KD mice. Equally, there were no differences between standard housed and enriched MSK1 KD mice. An RM-ANOVA showed a significant difference between standard housed and enriched WT mice, with enriched WT mice showing consistently greater PPF ( $F(6,112) = 5.71$ ;  $p = 0.019$ ), consistent with a reduced initial probability of neurotransmitter release. Insets show representative PPF profiles for each genotype and housing condition, normalised to the amplitude of the first of each pair of fEPSPs (broken grey line). Data are presented as mean  $\pm$  SEM

**Figure 5. MSK1 KD mice have greater spine density than WT mice.**

**A** MSK1 KD mice had higher spine density than WT mice, consistent with observations made previously (Corrêa et al., 2012); genotype effect  $F(1,283) = 14.55$ ,  $p = 0.000$ . Graph shows box plot of spine density distribution from 67-83 dendrites on CA1 neurons from 4 mice per condition. Box: 25-75% range; mean: horizontal line. Images show representative Golgi-stained CA1 secondary apical dendrites from across housing and genotype. Scale bars measure 10  $\mu\text{m}$ . **B** Graph shows cumulative distribution of spine density across housing and genotype. Note rightward shift in spine density distribution of MSK1 KD mutants (blue colours) reflecting the greater spine density shown in **A**.

**Figure 6. Experience enhances the dynamic range of synapses in an MSK1-dependent manner.**

1050 **A** Robust LTP was induced by theta burst stimulation in one pathway (at time 0, arrowhead; filled  
 1051 symbols) while the second pathway in each experiment (open symbols) remained unstimulated. LTP  
 1052 was enhanced exclusively in enriched WT slices. In the first 10 mins after LTP induction an ANOVA  
 1053 revealed no appreciable genotype x housing interaction ( $F(1,28) = 0.93$ ;  $p = 0.343$ ), but did show a  
 1054 significant effect of genotype ( $F(1,28) = 4.78$ ;  $p = 0.038$ ). A post-hoc analysis revealed LTP in the  
 1055 enriched WT group was significantly enhanced relative to enriched MSK1 KD mice ( $F(1,28) = 5.29$ ;  $p =$   
 1056  $0.029$ ). In the last hour of LTP (120 – 180 mins) an RM-ANOVA revealed a strong trend for a genotype  
 1057 x housing interaction ( $F(1,28) = 4.02$ ;  $p = 0.055$ ) in which LTP in the enriched WT group was  
 1058 significantly enhanced relative to enriched MSK1 KD mice ( $F(1,28) = 11.16$ ;  $p = 0.001$ ) and standard  
 1059 housed WT mice ( $F(1,28) = 8.37$ ;  $p = 0.007$ ). Insets are representative fEPSPs taken before (solid lines)  
 1060 and after (broken lines) LTP induction. Data shown as mean  $\pm$  SEM. **B** At time 0 LTD was induced by  
 1061 delivering 900 pulses at 1 Hz to one pathway (filled symbols), while the second pathway in each  
 1062 experiment (open symbols) remained unstimulated. A significant genotype x housing interaction was  
 1063 observed ( $F(1,24) = 4.47$ ;  $p = 0.045$ ) where LTD was enhanced in enriched WT mice relative to  
 1064 standard housed WT ( $F(1,24) = 13.52$ ;  $p = 0.001$ ) and enriched MSK1 KD mice ( $F(1,24) = 5.80$ ;  $p =$   
 1065  $0.024$ ). Insets are representative fEPSPs taken before (solid lines) and after (broken lines) LTD  
 1066 induction. Data shown as mean  $\pm$  SEM. **C** Cumulative distribution of changes of synaptic strength for  
 1067 each LTD and LTP experiment across housing and genotype. WT enriched mice show both left- (LTD)  
 1068 and rightward (LTP) shifts in the range of synaptic depression and potentiation, respectively,  
 1069 compared to standard housed WT mice. The distributions for standard house and enriched MSK1 KD  
 1070 mice overlap indicating their insensitivity to enrichment. **D** The net extent of the change in synaptic  
 1071 strength from baseline values (100 %) in each of the LTP (LTP % - 100 %) and LTD (100 % - LTD %)  
 1072 experiments shows a significant interaction between housing and genotype ( $F(1,56) = 4.58$ ;  $p = 0.037$ )

and a significant effect of enrichment only in WT mice ( $F(1,56) = 9.79$ ;  $p = 0.003$ ), and no effect in the MSK1 KD mutants ( $F(1,56) = 0.10$ ;  $p = 0.92$ ), where the dynamic range of synaptic plasticity remains unchanged after enrichment. Bar graph depicts mean  $\pm$  SEM and individual data points.

**Figure 7. Environmental enrichment influences gene expression in an MSK1-dependent manner.**

**A** Scree plot of principal component percentage variation for top 500 variance transcripts. The majority of the variance is explained by one principal component (73%), with the second principal component contributing 8% of the variance. **B** Principal component (PC) analysis of top 500 transcripts by variation across samples (solid symbols). Experimental groups are distinguished by color. Each group ( $n = 3$  to  $5$ ) is clustered around the arithmetic mean centroid (open symbol). The greatest variance (PC1; 73%) is explained by housing condition. **C** Schematic of differentially expressed gene (DEG) comparisons and number of differentially expressed genes between each genotype/housing condition combination (Tables 7-3 to 7-7). **D** Venn diagram comparing DEGs in response to enriched environment vs standard housed controls. Orange circle: DEGs in WT mice exposed to EE (475 genes; Extended Data Table 7-11 for Figure 7); Cyan circle: DEGs in MSK1 KD mice exposed to EE (256 genes; Extended Data Table 7-12 for Figure 7). **E, F** Back-to-back bar plots of significantly enriched GO Terms against Benjamini-adjusted  $p$  values (lilac bars), and the ratio of significant genes contributing to each term to the number expected in each category (yellow bars; the actual values for the number of significant and expected genes for each category are given). Broken vertical line at  $-2$  indicates  $p = 0.01$ . **E** Significantly enriched GO Terms common to both WT and MSK1 KD mice exposed to enrichment (214 genes). 14 of 152 significant categories are shown (Extended Data Table 7-8 for Figure 7). **F** Significantly enriched GO Terms unique to WT mice exposed to enrichment (261 genes). 6 of 7 unique categories out of 10 significant categories are shown (Extended Data Table 7-9 for Figure 7).

**Figure 8. Enrichment downregulates neurotrophin signaling-related genes in a MSK1-dependent manner.**

**A** MA plot of environmentally-enriched WT vs. environmentally-enriched MSK1 KD groups. DESeq2 beta-prior transformed log2 fold-changes are plotted on the y-axis. Broken lines at  $\pm 0.38$  equate to a  $\pm 1.3$ -fold change. Significantly differentially expressed genes are circled and labelled. **B** Heatmap of Z-score normalized rlog expression for genes identified as significantly different between MSK1 KD enriched (KD EE) and WT enriched (WT EE) groups (Extended Data Table 8-10 for Figure 8). Red cells indicate over-expression, blue cells indicate under-expression of a gene in a sample. Colors and labels at the top and bottom of the heatmap correspond to the sample's housing and genotype identity.

**Figure 9. MSK1 orchestrates homeostatic genomic downscaling of plasticity-related pathways and proteins in response to enrichment.**

**A** Heatmap of genes relevant to the BDNF/MAPK/MSK1 signaling cascade showing selective downregulation in samples from enriched WT mice (WT EE). For clarity the protein name is provided. For where the gene and protein name are not the same: TrkB2, Ntrk2; MEK2, Map2k2; MEK1, Map2k1; ERK1, Mapk3; ERK2, Mapk1; p38, Mapk14; MSK1, Rps6ka5; CREB, Creb1. **B, C** The expression of MSK1, but not MSK2, is selectively downregulated in response to enrichment (standard vs enriched housing; two-way ANOVA;  $F(3,13) = 29.01$ ;  $p = 0.0001$ ) in both WT ( $F(1,13) = 14.74$ ;  $p = 0.002$ ) and MSK1 KD mice ( $F(1,13) = 14.31$ ;  $p = 0.002$ ). Bar graph depicts mean  $\pm$  SEM and individual data points. **D** Western blot analysis of EGR1 protein in hippocampal tissue from separate samples contemporaneous with those used for RNA-Seq. There was a significant interaction genotype  $\times$  housing;  $F(3,12) = 7.17$ ,  $p = 0.020$  for EGR1 protein expression, which was selectively strongly downregulated in WT enriched mice ( $F(1,12) = 9.63$ ,  $p = 0.009$ ), in keeping with RNA-Seq DEG analysis (Figure 8A, B). Inset shows one of the two blots used in the analysis of EGR1 expression. WE, WT

1119 enriched (n = 4); WS, WT standard housed (n = 4); KE, MSK1 KD enriched (n = 4); KS, MSK1 KD  
1120 standard housed (n = 4). Bar graph depicts mean  $\pm$  SEM and individual data points. **E** Western blot  
1121 analysis of Arc/Arg3.1 protein in hippocampal tissue from separate samples contemporaneous with  
1122 those used for RNA-Seq. There was a significant interaction genotype x housing;  $F(3,12) = 9.95$ ,  $p =$   
1123  $0.008$  for Arc/Arg3.1 protein expression, which was selectively strongly downregulated in WT  
1124 enriched mice ( $F(1,12) = 7.64$ ,  $p = 0.017$ ). Inset shows one of the two blots used in the analysis of  
1125 Arc/Arg3.1 expression. WE, WT enriched (n = 4); WS, WT standard housed (n = 4); KE, MSK1 KD  
1126 enriched (n = 4); KS, MSK1 KD standard housed (n = 4). Bar graph depicts mean  $\pm$  SEM and individual  
1127 data points.





















